Q5® Site- Directed Mutagenesis Kit Protocol

Step I: PCR

1.Assemble the following reagents in a PCR tube:

1. 12,5µl Q5 Hot Start High- Fidelity 2X Master Mix
2. 1,25 µl Forward Primer
3. 1,25 µl Reverse Primer
4. 1µl Template (1- 25ng/µl)
5. 9µl nuclease- free water

2. Thermocycling conditions for a Routine PCR:

1. Initial Denaturation: 30s at 98°C
2. 25 Cycles :
    a. 10s at 98°C
    b. 30s at Ta°C
    c. 30s/kb at 72°C
3. Final Extension: 2 minutes at 72°C

Step II: Kinase, Ligase & DpnI (KLD) Treatment

1.Assemble the following reagents:

1. 1µl PCR Product
2. 5µl 2X KLD Reaction Buffer
3. 1µl 10X KLD Enzyme Mix
4. 3µl Nuclease-free Water

2.Mix by pipetting up and down, incubate at room temperature for 5 minutes

Step III: Transformation

1. Thaw 50 µl competent cells on ice (15-20 min)
2. Mix 5 µl of ligation with the cells
3. Incubate on ice for 30 min
4. Heat shock at 42 °C for 30-60 s
5. Incubate on ice for 3 min
6. Pipette 800 µl LB medium
7. Incubate at 37 °C for 70 min on shaker
8. Plate on appropiate antibiotic medium

Step II: Kinase, Ligase & DpnI (KLD) Treatment

1. Assemble the following reagents:

	VOLUME	FINAL CONC.
PCR Product	1 µl	
2X KLD Reaction Buffer	5 µl	1X
10X KLD Enzyme Mix	1 µl	1X
Nuclease-free Water	3 µl	

2. Mix well by pipetting up and down and incubate at room temperature for 5 minutes.

Step III: Transformation

1. Thaw a tube of NEB 5-alpha Competent *E. coli* cells on ice.
2. Add 5 µl of the KLD mix from Step II to the tube of thawed cells. Carefully flick the tube 4-5 times to mix. Do not vortex.
3. Place the mixture on ice for 30 minutes.
4. Heat shock at 42°C for 30 seconds.
5. Place on ice for 5 minutes.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Incubate at 37°C for 60 minutes with shaking (250 rpm).
8. Mix the cells thoroughly by flicking the tube and inverting, then spread 50-100 µl onto a selection plate and incubate overnight at 37°C. It may be necessary (particularly for simple substitution and deletion experiments) to make a 10- to 40-fold dilution of the transformation mix in SOC prior to plating, to avoid a lawn of colonies